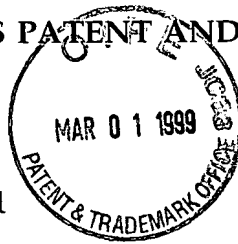


# 9200

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 5,057,540  
Issued: October 15, 1991  
In the Name of: Charlotte A. Kensil and Dante J. Marciani  
For: Saponin Adjuvant  
Docket No.: 106941.141



CobC  
R

**REVIEW**

TRANSMITTAL LETTER

Assistant Commissioner for Patents  
Washington, DC 20231

**CERTIFICATE**

**MAR 16 1999**

Sir:

**OF CORRECTION**

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C. 20231.

on Feb. 23, 1999  
(Date of Deposit)  
Karen Kenney  
Person Making Deposit  
Km [Signature]  
Signature

The following documents are submitted for filing:

1. Request for a Certificate of Correction Under 37 C.F.R. § 1.323;
2. A copy of the April, 1985 "VYDAC HPLC COLUMNS AND PACKING MATERIALS" catalog from The Separations Group;
3. Certificate of Correction; and
4. Return Postcard.

The Commissioner is authorized to debit the fee set forth in 37 C.F.R. § 1.20(a) of \$100.00 from Deposit Account No. 08-0219. No other fee is believed to be due, however, if one is required, the Commissioner is authorized to debit such fee from Deposit Account No. 08-0219.

Dated: February 23, 1999

Respectfully submitted,

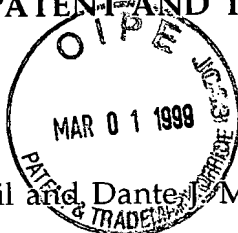
*Colleen Superko*

Hale and Dorr LLP  
60 State Street  
Boston, MA 02109  
(617) 526-6000

Colleen Superko  
Registration No. 39,850

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 5,057,540  
Issued: October 15, 1991  
In the Name of: Charlotte A. Kensil and Dante Marciani  
For: Saponin Adjuvant  
Docket No.: 106941.141



**REQUEST FOR A CERTIFICATE OF CORRECTION**  
**UNDER 37 C.F.R. § 1.323**

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

The attached Certificate of Correction is made under 37 C.F.R. § 1.323 and is respectfully requested to be issued under 35 U.S.C. § 255.

In this Request for a Certificate of Correction, it shows that mistakes occurred in good faith and do not involve such changes in the patent as would either constitute new matter or would require reexamination.

The Commissioner is authorized to debit the fee set forth in 37 C.F.R. § 1.20(a) of \$100.00 from Deposit Account No. 08-0219. No other fee is believed to be due, however, if one is required, the Commissioner is authorized to debit such fee from Deposit Account No. 08-0219.

This Request for a Certificate of Correction is to correct the pore size for the Vydac C<sub>4</sub> columns to 300 Å and to correct the pseudo-molecular ion mass of QA-21 to 1988.

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C. 20231.

on Feb. 23, 1999  
(Date of Deposit)

Karen Kenney  
Person Making Deposit

[Signature]  
Signature

U.S. Patent No. 5,057,540  
Request for a Certificate of Correction

This request is being made for the following reasons:

The Vydac C<sub>4</sub> columns referenced in the specification and in the claims are commercially available reverse phase high pressure liquid chromatography ("HPLC") columns from The Separations Group, Hesperia, California. In addition, "Vydac" is a trademark of The Separations Group.

To support this Request for a Certificate of Correction, attached is a copy of the "VYDAC HPLC COLUMNS AND PACKING MATERIALS" catalog from The Separations Group, dated April, 1985. The catalog lists the complete line of HPLC columns that were commercially available at the time the underlying patent application was filed. On page 1 of the catalog is an index identifying the Vydac C<sub>4</sub> reverse phase HPLC columns as product "214 TP: Wide pore C<sub>4</sub> Reverse Phase" and indicating pages 8-9 of the catalog for a description. On page 8 of the catalog, the Vydac C<sub>4</sub> column is described as "[a] large pore (300 Å) spheroidal column packing material - ideal for large biomolecules - with an endcapped C-4 reverse phase." On page 9 of the catalog, the table shows that there are eleven different columns sold as Vydac C<sub>4</sub> columns, all with the same 300 Å pore size, but with different particle sizes, different column interior diameters ("i.d."), and different lengths.

There are two different kinds of Vydac C<sub>4</sub> columns referenced in the instant specification and one kind of Vydac C<sub>4</sub> column referenced in the claims. In the specification, a preparative Vydac C<sub>4</sub> column has a 5 µm particle size, 10 mm column i.d.

U.S. Patent No. 5,057,540  
Request for a Certificate of Correction

and 25 cm length. An analytical Vydac C<sub>4</sub> column having a 5  $\mu$ m particle size, 4.6 mm column i.d., and 25 cm length is the second type described in the specification. In the claims, reference is made only to the analytical Vydac C<sub>4</sub> column, that is a Vydac C<sub>4</sub> column having 5  $\mu$ m particle size, 4.6 mm column i.d., and 25 cm length.

Thus, it is clear that any Vydac C<sub>4</sub> column always has a 300 Å pore size. For identification purposes, then, the patent could have simply listed the Vydac C<sub>4</sub> columns as a 5  $\mu$ m particle size, 4.6 mm column i.d. (or 10 mm column i.d.) and 25 cm length. The 300 Å pore size is not a necessary characteristic to be listed as, by definition from the manufacturer, all Vydac C<sub>4</sub> columns have a 300 Å pore size.

In the specification and the claims, all references, except one, as noted below, erroneously give the pore size of the Vydac C<sub>4</sub> columns as 330 Å. As pointed out above, the correct pore size for the Vydac C<sub>4</sub> reverse phase HPLC columns is 300 Å.

The one reference to the correct pore size for the Vydac C<sub>4</sub> column is made in the patent specification at column 16, the sixth line under TABLE 4. Here, the HPLC conditions are provided as "Vydac [C<sub>4</sub>], 5  $\mu$ m particle size, 300 Å pore size, .46 x 25 cm."

Another error occurred with regard to the pseudo-molecular ion mass of QA-21. The specification erroneously refers to the pseudo-molecular ion mass of QA-21 as 1980. The correct pseudo-molecular ion mass for QA-21 is 1988. The correct ion mass of QA-21 is found in the accompanying figures. Figure 8C, which is sheet 16 of 23 in the patent, provides the mass spectroscopy fast action bombardment of QA-21 and indicates,

U.S. Patent No. 5,057,540  
Request for a Certificate of Correction

on the far right hand side, that the appropriate pseudo-molecular ion mass of QA-21 is 1988.

Hence, the errors in providing the pore size for the Vydac C<sub>4</sub> column as 300 Å and in providing the pseudo-molecular ion mass of QA-21 as 1980 were typographical errors. The errors were made in the first draft of the patent application, with the exceptions noted, and continued to be made throughout the prosecution of the application. The errors were made in good faith and without deceptive intent. Further, as shown above, correcting the pore size and the pseudo-molecular ion mass mistakes are of minor character that do not involve a change in the patent that would either constitute new matter or would require reexamination.

Although the 300 Å pore size was correctly given with TABLE 4 and the 1988 ion mass was correctly given in Figure 8C, the errors of the incorrect descriptions of a 330 Å pore size and of the 1980 ion mass were not noticed until it was first brought to the attention of the patent assignee in a European Opposition of the corresponding European Patent. The errors were made in good faith and without deceptive intent.

In conclusion, correcting the pore size from 330 Å to 300 Å and the pseudo-molecular mass of QA-21 from 1980 to 1988 are mistakes of minor character, that are not the fault of the Patent and Trademark Office, and a showing has been made that such mistakes occurred in good faith and without deceptive intent. Again, the corrections do



U.S. Patent No. 5,057,540  
Request for a Certificate of Correction

not involve such changes in the patent as would either constitute new matter or would require reexamination.

The Commissioner is therefore respectfully requested to issue the attached Certificate of Correction.

Respectfully submitted,

A handwritten signature in cursive script that reads "Colleen Superko".

Colleen Superko  
Registration No. 39,850

Dated: February 23, 1999

Hale and Dorr LLP  
60 State Street  
Boston, MA 02109  
(617) 526-6000

/netuser6/karenb/baker/aquila/106941.141/certcorr.wpf

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO.: 5,057,540  
DATED: October 15, 1991  
INVENTORS: Charlotte A. Kensil and Dante J. Marciani

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At column 4, line 53, "330 Å" should be -- 300 Å --.  
At column 5, line 66, "330 Å" should be -- 300 Å --.  
At column 6, line 2, "330 Å" should be -- 300 Å --.  
At column 6, line 18, "330 Å" should be -- 300 Å --.  
At column 6, line 22, "330 Å" should be -- 300 Å --.  
At column 6, line 39, "330 Å" should be -- 300 Å --.  
At column 6, line 43, "330 Å" should be -- 300 Å --.  
At column 6, line 59, "330 Å" should be -- 300 Å --.  
At column 6, line 62, "330" should be -- 300 --.  
At column 9, line 43, "330 Å" should be -- 300 Å --.  
At column 10, line 31, "330 Å" should be -- 300 Å --.  
At column 12, line 56, "1980" should be -- 1988 --.  
In claim 1, at column 22, line 63, "330 Å" should be -- 300 Å --.  
In claim 2, at column 23, line 8, "330 Å" should be -- 300 Å --.  
In claim 5, at column 23, line 33, "330 Å" should be -- 300 Å --.  
In claim 8, at column 23, line 59, "330 Å" should be -- 300 Å --.  
In claim 11, at column 24, line 25, "330 Å" should be -- 300 Å --.

MAILING ADDRESS OF SENDER  
Colleen Superko  
Hale and Dorr LLP  
60 State Street  
Boston, Massachusetts 02109

PATENT NO. 5,057,540



**WYDA**

# HPLC COLUMNS AND PACKING MATERIALS

**THE SEP/A/RA/TIONS GROUP**

P. O. Box 867  
Hesperia, California 92345  
(619) 244-6107

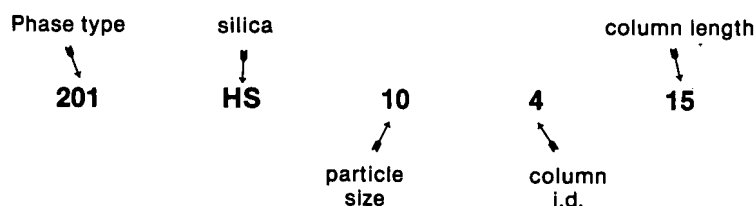
**APRIL 1985**



About the covers...

Vydac High-Performance Liquid Chromatography columns and packing materials are manufactured in a quiet, beautiful location in the California high desert typified by the abundant Joshua trees unique to this location.

Vydac products are described by a catalog number such as the following:



This catalog number describes a column packed with reverse phase 201 on 10 micron HS silica, 4.6 mm in diameter (only the integer is used) and fifteen cm in length (catalog numbers for 25 cm columns omit the column length as this is standard). Catalog numbers for bulk materials have a 'B' after the silica type and no column dimensions: an example is '201HSB10' to describe bulk 201 reverse phase on 10 micron HS silica.

Vydac catalog numbers designate the bonded phase as:

100 series .....	Unbonded silica
200 series .....	Reverse phase
300 series .....	Anion exchange
400 series .....	Cation exchange
500 series .....	Cyano polar phase
600 series .....	Amino polar phase

Vydac columns feature high-quality Valco inverted endfittings.

The Separations Group is the leading manufacturer of high purity HPLC spheroidal silica in the world today with fourteen years experience providing quality HPLC columns to the analytical community. Our **Vydac** HPLC columns are *unique*—just like our California high desert location. We manufacture our own **high purity silicas**, carefully size them into narrow particle size ranges and chemically bond various chromatographic phases. Each batch of material is carefully tested for selectivity and packing efficiency after which columns are packed by proprietary methods. Every column is individually tested to assure the highest quality final product. These steps combine to produce exceptional columns with unique separation characteristics. **Vydac** columns and packing materials stand out from the crowd and are frequently the choice of knowledgeable scientists in their respective application fields.

**Vydac** is a trademark of The Separations Group

<b>INDEX</b>	
Product	Page No.
Silica substrates	2-3
201 HS: High Capacity Reverse Phase	4-5
201 TP: Medium capacity Reverse Phase	6-7
214 TP: Wide pore C <sub>18</sub> Reverse Phase	8-9
218 TP: Wide pore C <sub>18</sub> Reverse Phase	10-11
219 TP: Wide pore phenyl Reverse Phase	12-13
Preparative HPLC	14-15
Ion chromatography	16-17
Ion exchange	18
Solid Core (Pellicular)/Pre-column kits	19
Selected Bibliography	20-21

For analytical to preparative separations...

**Vydac** columns are available in three diameters: analytical (4.6 mm), semi-preparative (10 mm) and preparative (22 mm). Bulk packing material is available in 10 micron, 15-20 micron and 20-30 micron particle sizes of the same silica substrate and using the same bonding chemistry, permitting direct scale-up of analytical separations.

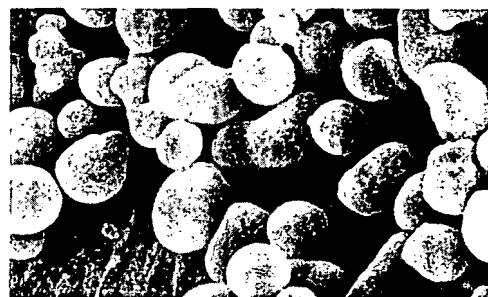
# **The beginning...**

## **High Purity Silica**

The Separations Group manufactures two types of high purity silica for high-performance liquid chromatography:

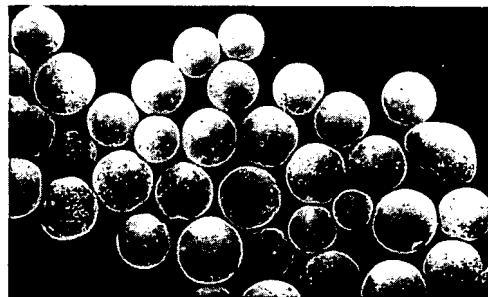
### **Vydac TP Silica**

TP silica is spheroidal, with 300 angstrom pores, a low surface area of 80 sq. m. per gram and a pore volume of .6 cc/gram. The wide pores in TP silica make it ideal for the separation of large bio-molecules. Its low surface area results in a medium capacity reverse phase, making it an excellent material for the chromatography of very hydrophobic small molecules.



### **Vydac HS Silica**

HS silica is spherical, with 80 angstrom pores, a surface area of 500 sq. m. per gm and a pore volume of .8 cc/gram. The high surface area of HS silica results in a high loading of bonded phase making this material particularly good for the separation of relatively hydrophilic compounds.



Both TP and HS silica are physically rugged and offer stable, reproducible columns with moderate backpressure even with small particles. The Separations Group has developed a family of quality HPLC separation products based on these high purity silicas. They are excellent substrates to which many chromatographic phases may be bonded. The combination of high purity silica and special bonding techniques yield unique separation materials.

The manufacturing process for Vydac TP and HS silica yields:

- **High purity silica, low in metal and sulphate content and free from extraneous chromatographic effects**
- **Rigid, physically rugged, spheroidal particles that do not fracture in the column thus reducing the backpressure and lengthening the lifetime of High-Performance Liquid Chromatography columns**
- **Reproducible materials that give the same separations column after column. Careful quality control assures the chromatographer of reliable results.**

Vydac unbonded TP and HS silicas are available in columns and bulk:

**COLUMNS:**

Catalog No.	Particle Size	Column I.d.	Length	Price
101HS54	5 micron	4.6 mm	25 cm	\$ 250
101HS104	10 micron	4.6 mm	25 cm	209
101TP54	5 micron	4.6 mm	25 cm	250
101TP104	10 micron	4.6 mm	25 cm	209

**BULK:**

Catalog No.	Particle Size	Price / gram			
		10 gm	100 gm	500 gm	1 kilogram
101HSB10	10 micron	13.20	10.80	8.90	8.60
101HSB1520	15-20 micron	---	2.50	2.50	1.75
101HSB2030	20-30 micron	---	2.00	1.50	1.30
101TPB10	10 micron	13.20	10.80	8.90	8.60
101TPB1520	15-20 micron	---	2.50	2.50	1.75
101TPB2030	20-30 micron	---	2.00	1.50	1.30

Bulk packing materials are available in larger quantities and other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

# Vydac 201 HS Reverse Phase

201 HS is a high capacity reverse phase material. An eighteen carbon chain is chemically bonded to the high surface area HS silica which is then endcapped with trimethylsilane. 201HS columns and packing materials feature:

- A high surface area, spherical silica
- A high loading of the bonded phase resulting in a high capacity endcapped, C-18 reverse phase material
- Excellent separations for relatively polar compounds such as the B vitamins, organic acids and biological bases and nucleosides

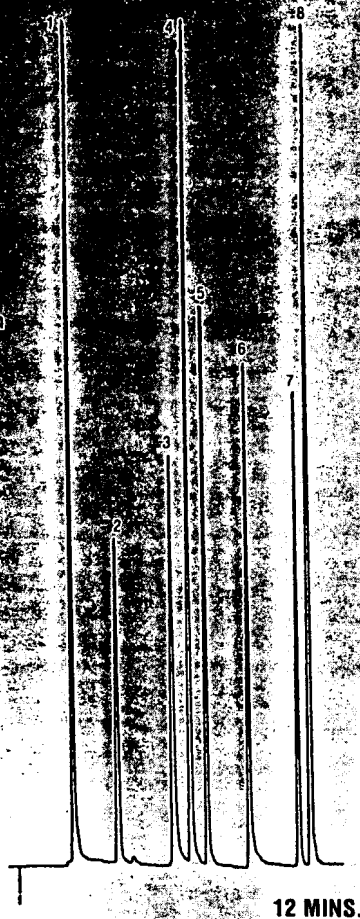
## Separation of B Vitamins

### CONDITIONS

Solvent #1: 0.1M KOAc adj. to pH 4.7  
with formic acid  
Solvent #2: 50% acetonitrile: 50 % water  
Start: 95% Solvent #1, 5% Solvent #2  
Linear gradient programmed  
over 15 min. to 100% Solvent #2  
Flowrate: 1.5 ml/min  
Detector: UV @ 254 nm.

### PEAK IDENTITY

1. Vitamin C - Ascorbic Acid
2. Nicotinic Acid - Niacin
3. Vitamin B<sub>6</sub> - Pyridoxine
4. Vitamin B<sub>1</sub> - Thiamine
5. Vitamin B<sub>3</sub> - Nicotinamide
6. Vitamin M - Folic Acid
7. Vitamin B<sub>12</sub> - Cyanocobalamin
8. Vitamin B<sub>2</sub> - Riboflavin



## Separation of Organic Acids

### CONDITIONS

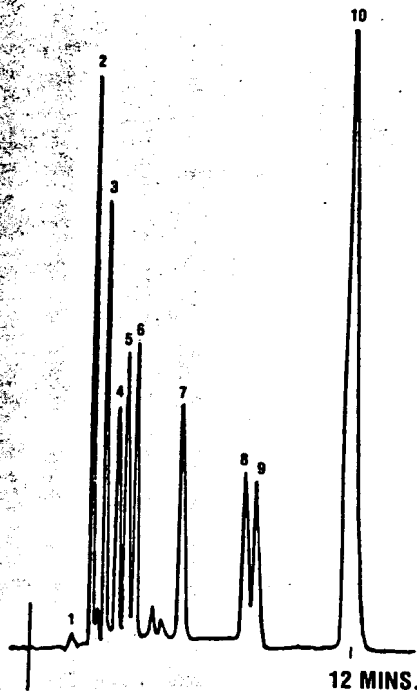
Solvent: .01M Triethylamine  
adj. to pH 2.0  
Phosphoric Acid

Flowrate: 1.5 ml/min

Detector: UV @ 220 nm.

### PEAK IDENTITY

- |                   |                   |
|-------------------|-------------------|
| 1. Solvent front  | 6. Acetic Acid    |
| 2. Glyoxalic Acid | 7. Citric Acid    |
| 3. Tartaric Acid  | 8. Succinic Acid  |
| 4. Malic Acid     | 9. Propionic Acid |
| 5. Lactic Acid    | 10. Maleic Acid   |



Best Available Copy

# ANALYSIS OF NUCLEOSIDES AND BASES

## PEAK IDENTITY

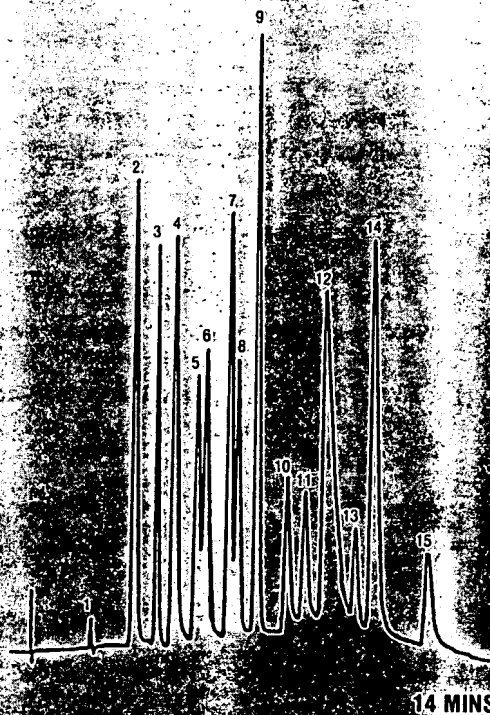
1. Solvent
2. Cytosine
3. Uracil
4. Cytidine
5. 5-methyl cytosine
6. Uridine
7. Hypoxanthine
8. Guanine
9. Thymine
10. Inosine
11. Guanosine
12. Xanthine
13. Thymidine
14. Adenine
15. Adenosine

## CONDITIONS

Eluent: A: 01 M KOAc pH 5.0

B: 25:75 ACN:H<sub>2</sub>O

Gradient: 5 to 100% B in 30 min



Vydac 201 HS is available in pre-packed columns and in bulk:

### COLUMNS:

Catalog No.	Particle Size	Column I.d.	Length	Price
<b>ANALYTICAL</b>				
201HS5415	5 micron	4.6 mm	15 cm	\$ 300
201HS54	5 micron	4.6 mm	25 cm	325
201HS10415	10 micron	4.6 mm	15 cm	225
201HS104	10 micron	4.6 mm	25 cm	248
<b>PREPARATIVE</b>				
201HS510	5 micron	10 mm	25 cm	800
201HS1010	10 micron	10 mm	25 cm	600
201HS1022	10 micron	22 mm	25 cm	1600

### BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
201HSB10	10 micron	19.30	15.00	12.00	11.00
201HSB1520	15-20 micron	---	3.00	3.00	2.00
201HSB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities and other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

# Vydac 201 TP Reverse Phase

201 TP is a medium capacity reverse phase material. An eighteen carbon chain is chemically bonded to the low surface area TP silica resulting in a medium level phase loading. 201 TP columns and packing materials feature:

- **A low surface area, spheroidal silica**
- **A moderate loading of bonded phase resulting in a medium capacity non-encapped, C-18 reverse phase material**
- **Excellent separations for very non-polar compounds such as polynuclear aromatic hydrocarbons, aflatoxins and alpha and beta carotenes.**

## ANALYSIS OF POLYAROMATIC HYDROCARBONS

### CONDITIONS

Gradient:

Solvent #1: H<sub>2</sub>O

Solvent #2: Acetonitrile

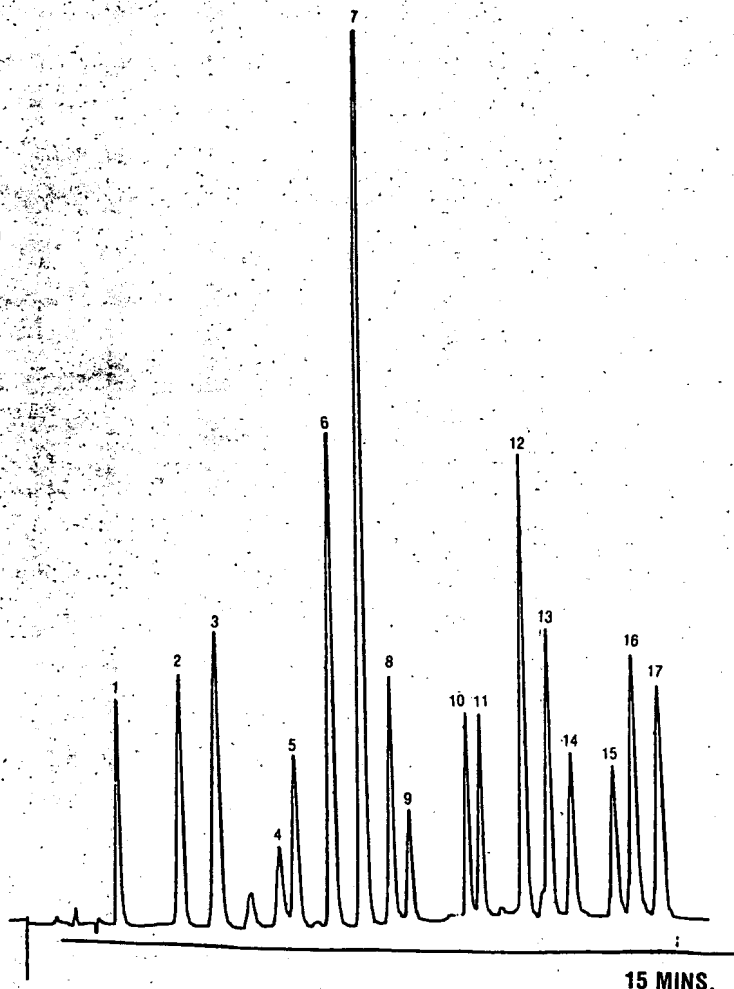
Start 50% solvent #2 for 3 minutes then run Linear Gradient programmed over 7 minutes to 100% solvent #2.

Flowrate: 1.5 ml/min

Detector: UV @ 254 nm.

### PEAK IDENTITY

1. Benzene
2. Naphthalene
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benz (a) anthracene
11. Chrysene
12. Benzo (b) fluoranthene
13. Benzo (k) fluoranthene
14. Benz (a) pyrene
15. Dibenz (a, h) anthracene
16. Benzo (g, h, i) perylene
17. Indeno (1, 2, 3, c, d) pyrene



## ALPHA AND BETA CAROTENES

Eluent: 90% Acetonitrile/10% Chloroform

Flowrate: 1.5 ml/min

Detector: UV @ 254 nm.

## PEAK IDENTITY

1. Alpha-Carotene

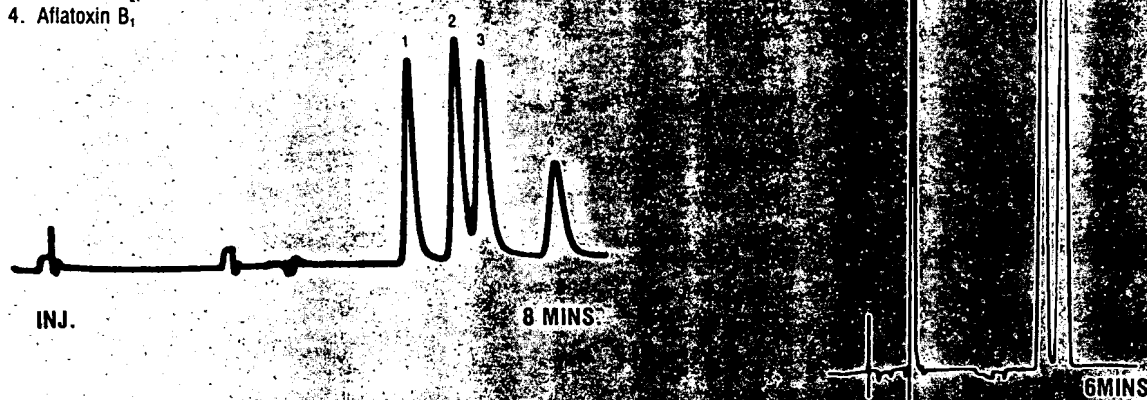
2. Beta-Carotene

## AFLATOXINS

Eluent: 100:20:20

H<sub>2</sub>O:ACN:MeOH

1. Aflatoxin G<sub>2</sub>
2. Aflatoxin G<sub>1</sub>
3. Aflatoxin B<sub>2</sub>
4. Aflatoxin B<sub>1</sub>



Vydac 201TP is available in pre-packed columns and in bulk:

## COLUMNS:

Catalog No.	Particle Size	Column i.d.	Length	Price
<b>ANALYTICAL</b>				
201TP5415	5 micron	4.6 mm	15 cm	\$ 300
201TP54	5 micron	4.6 mm	25 cm	325
201TP10415	10 micron	4.6 mm	15 cm	225
201TP104	10 micron	4.6 mm	25 cm	248
<b>PREPARATIVE</b>				
201TP510	5 micron	10 mm	25 cm	800
201TP1010	10 micron	10 mm	25 cm	600
201TP1022	10 micron	22 mm	25 cm	1600

## BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
201TPB10	10 micron	19.30	15.00	12.00	11.00
201TPB1520	15-20 micron	---	3.00	3.00	2.00
201TPB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities or other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.



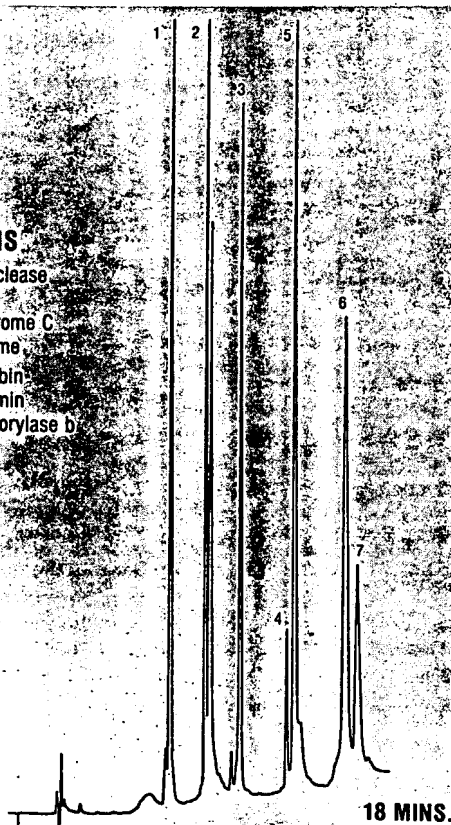
# Vydac 214 TP Reverse Phase

214 TP is a short chain reverse phase material consisting of a four carbon chain chemically bonded to low surface area TP silica which is then endcapped with trimethylsilane to prevent adsorption of polar compounds. 214 TP columns and packing materials feature:

- **A premium column packing material—the choice of many bioscientists for their most demanding protein and peptide separations**
- **A large pore (300 Å) spheroidal column packing material—ideal for large biomolecules—with an endcapped C-4 reverse phase**
- **High purity silica with few free silanol groups resulting in good recovery for most proteins and peptides**
- **Excellent separations of peptides and proteins.**

## PROTEINS

1. Ribonuclease
2. Insulin
3. Cytochrome C
4. Lysozyme
5. Myoglobin
6. Ovalbumin
7. Phosphorylase b

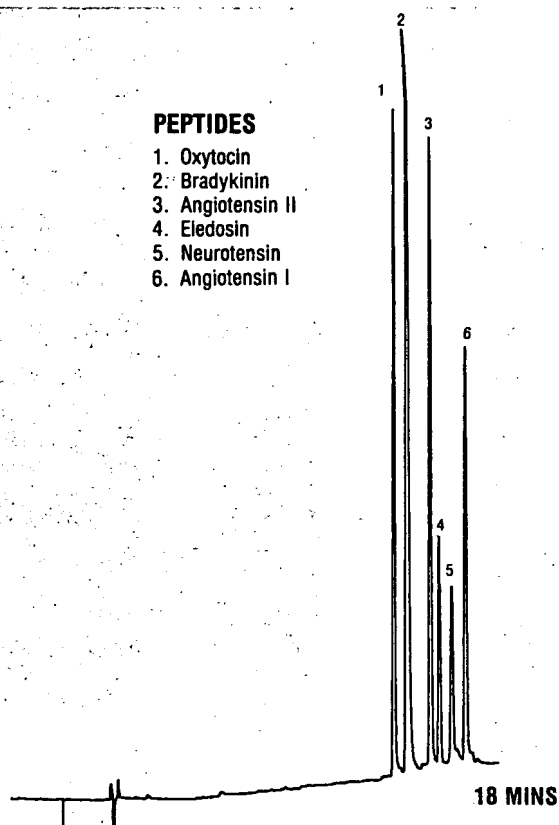


Solvent a: .1% TFA in H<sub>2</sub>O  
 b: .1% TFA in 95/5 ACN/H<sub>2</sub>O  
 Gradient: 25% B to 100% B over 30 min  
 Flowrate: 1.5 ml/min

## CONDITIONS

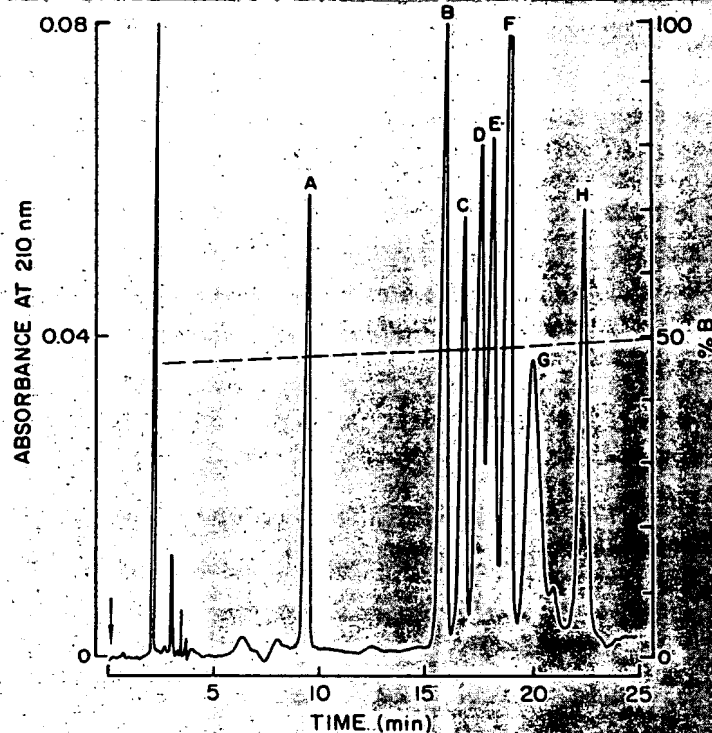
## PEPTIDES

1. Oxytocin
2. Bradykinin
3. Angiotensin II
4. Eledosin
5. Neurotensin
6. Angiotensin I



Solvent a: .1% TFA in H<sub>2</sub>O  
 b: .1% TFA in 30/70 H<sub>2</sub>O/ACN  
 Gradient: 5% B to 100% B over 30 min  
 Flowrate: 1.5 ml/min

## SEPARATION OF INSULINS



Insulins: A) chicken, B) bovine, C) ovine, D) rabbit,  
E) human, F) porcine, G) rat I, H) rat II

Eluents: A: .1% TFA in H<sub>2</sub>O B: .1% TFA in ACN:H<sub>2</sub>O (60:40)

Gradient: 45% to 50% B in 25 min

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Vydac 214 TP is available in pre-packed columns and in bulk:

## COLUMNS:

Catalog No.	Particle Size	Column i.d.	Length	Price
<b>ANALYTICAL</b>				
214TP5415	5 micron	4.6 mm	15 cm	\$ 300
214TP54	5 micron	4.6 mm	25 cm	325
214TP10415	10 micron	4.6 mm	25 cm	225
214TP104	10 micron	4.6 mm	25 cm	248

## PREPARATIVE

214TP510	5 micron	10 mm	25 cm	800
214 TP1010	10 micron	10 mm	25 cm	600
214TP1022	10 micron	22 mm	25 cm	1600
214TP152022	15-20 micron	22 mm	25 cm	995

## BULK:

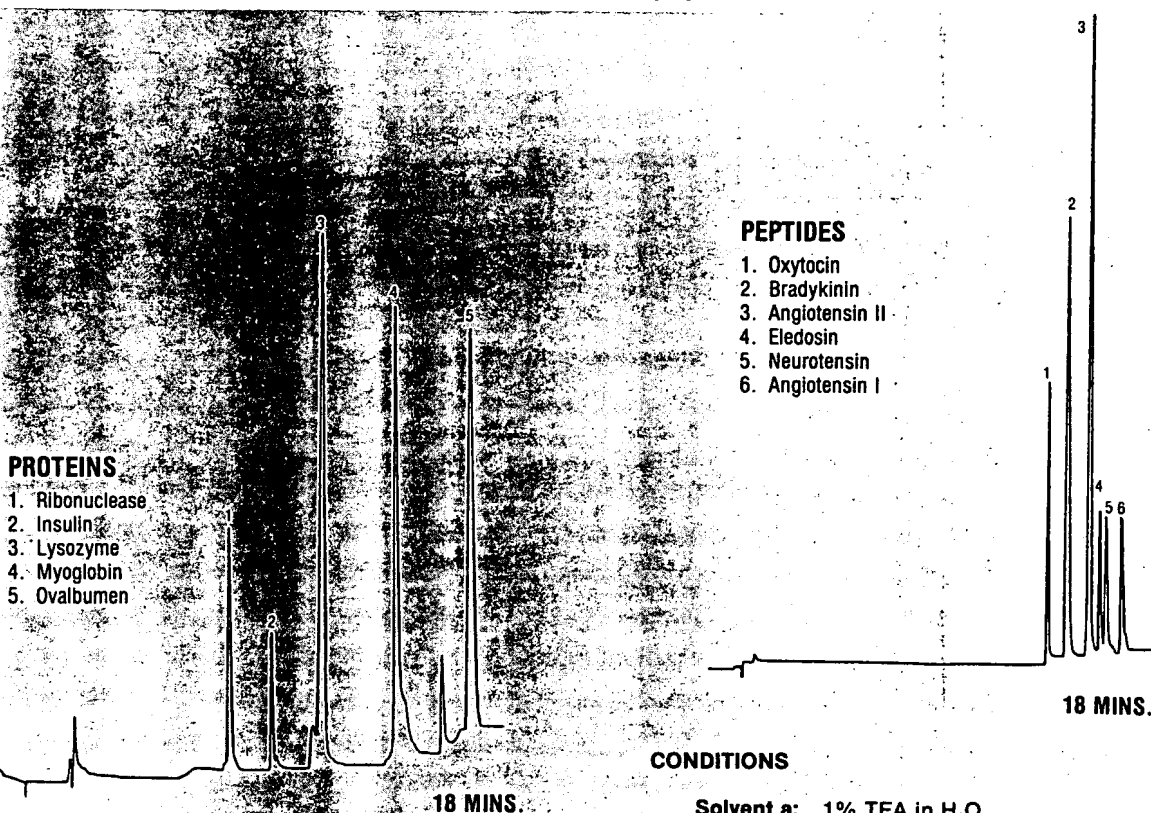
Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
214TPB10	10 micron	19.30	15.00	12.00	11.00
214TPB1520	15-20 micron	---	3.00	3.00	2.00
214TPB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities or other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

# Vydac 218 TP Reverse Phase

218 TP is a long chain reverse phase material consisting of an eighteen carbon chain chemically bonded to low surface area TP silica which is then endcapped with trimethylsilane to prevent adsorption of polar compounds. 218 TP columns and packing materials feature:

- **A premium column packing material—together with its companion, 214 TP, the choice of many bioscientists for their most demanding protein and peptide separations**
- **A large pore (300Å) spheroidal column packing material—ideal for large biomolecules—with an endcapped C-18 reverse phase**
- **High purity silica with few free silanol groups resulting in good recovery for most proteins and peptides**
- **Excellent separations of proteins and peptides**



## CONDITIONS

Solvent a: .1% TFA in H<sub>2</sub>O  
b: .1% TFA in 95/5 ACN/H<sub>2</sub>O  
Gradient: 25% B to 100% B over 30 min  
Flowrate: 1.5 ml/min

## CONDITIONS

Solvent a: .1% TFA in H<sub>2</sub>O  
b: .1% TFA in 30/70 H<sub>2</sub>O/ACN  
Gradient: 5% to 100% B over 30 min  
Flowrate: 1.5 ml/min

# TRYPTIC DIGEST OF CYTOCHROME c

## CONDITIONS

### Solvent Gradient

Solvent #1: 0.1% TFA in water

Solvent #2 0.1% TFA in 70% acetonitrile/  
30% water

Linear Gradient programmed over 2 hours  
from 100% of solvent #1 to 100% solvent #2

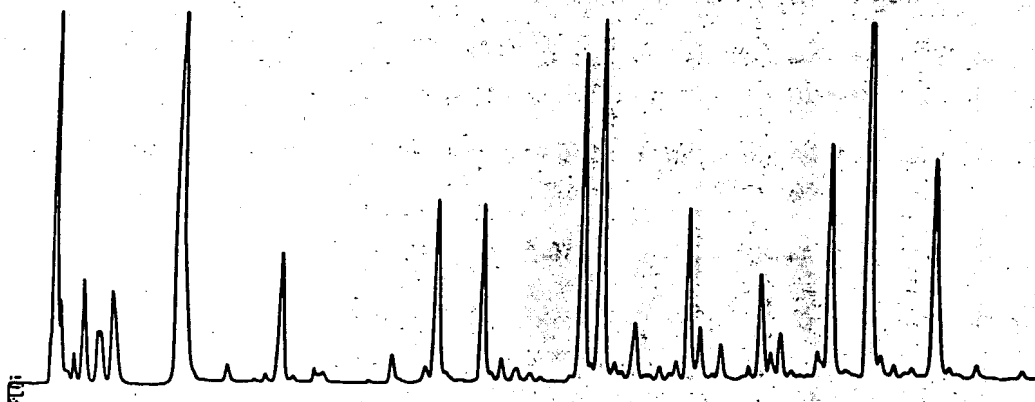
Flowrate: 1-ml/min

Detector: UV@ 214 nm

Cytochrome C Tryptic Digest-30 micrograms

Courtesy of:

Dr. Brian Clark  
City of Hope Research Institute  
Division of Immunology  
Duarte, CA 91010



Vydac 218 TP is available in pre-packed columns and in bulk:

## COLUMNS:

Catalog No.	Particle Size	Column I.d.	Length	Price
<b>ANALYTICAL</b>				
218TP5415	5 micron	4.6 mm	15 cm	\$ 300
218TP54	5 micron	4.6 mm	25 cm	325
218TP10415	10 micron	4.6 mm	15 cm	225
218TP104	10 micron	4.6 mm	25 cm	248
<b>PREPARATIVE</b>				
218TP510	5 micron	10 mm	25 cm	800
218TP1010	10 micron	10 mm	25 cm	600
218TP1022	10 micron	22 mm	25 cm	1600
218TP152022	15-20 micron	22 mm	25 cm	995

## BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
218TPB10	10 micron	19.30	15.00	12.00	11.00
218TPB1520	15-20 micron	---	3.00	3.00	2.00
218TPB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities or other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

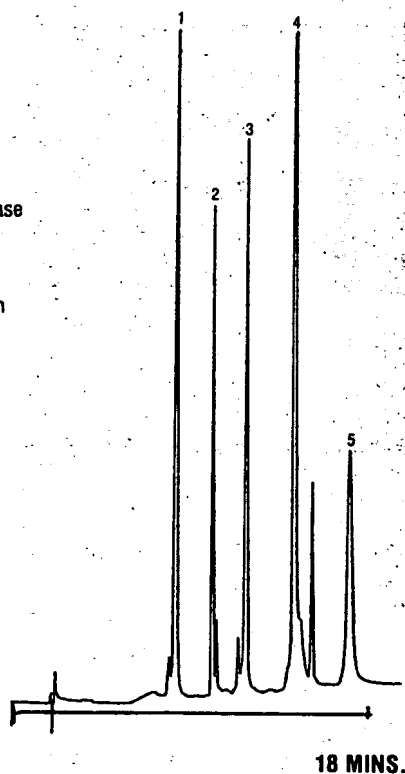
# Vydac 219 TP Reverse Phase

219 TP is an aromatic reverse phase material consisting of a diphenyl group chemically bonded to low surface area TP silica which is then endcapped with trimethylsilane to prevent adsorption of polar compounds. 219 TP columns and packing material feature:

- **An alternative selectivity for proteins and peptides by means of the aromatic diphenyl reverse phase**
- **A large pore (300 Å) spheroidal column packing material—ideal for large biomolecules**
- **High purity silica with few free silanol groups resulting in good recovery for most proteins and peptides**
- **Excellent separations of proteins and peptides**

## PROTEINS

1. Ribonuclease
2. Insulin
3. Lysozyme
4. Myoglobin
5. Ovalbumin

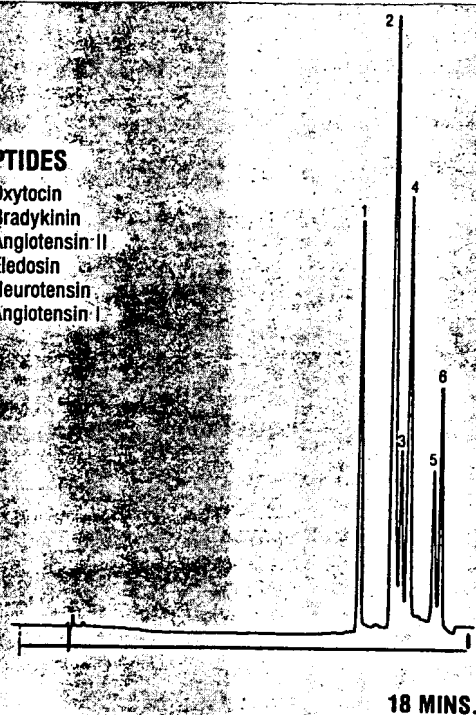


## CONDITIONS

Solvent a: .1% TFA in H<sub>2</sub>O  
 b: .1% TFA in 95/5 ACN/H<sub>2</sub>O  
 Gradient: 25% B to 100% B over 30 min  
 Flowrate: 1.5 ml/min

## PEPTIDES

1. Oxytocin
2. Bradykinin
3. Angiotensin II
4. Eledosin
5. Neurotensin
6. Angiotensin I

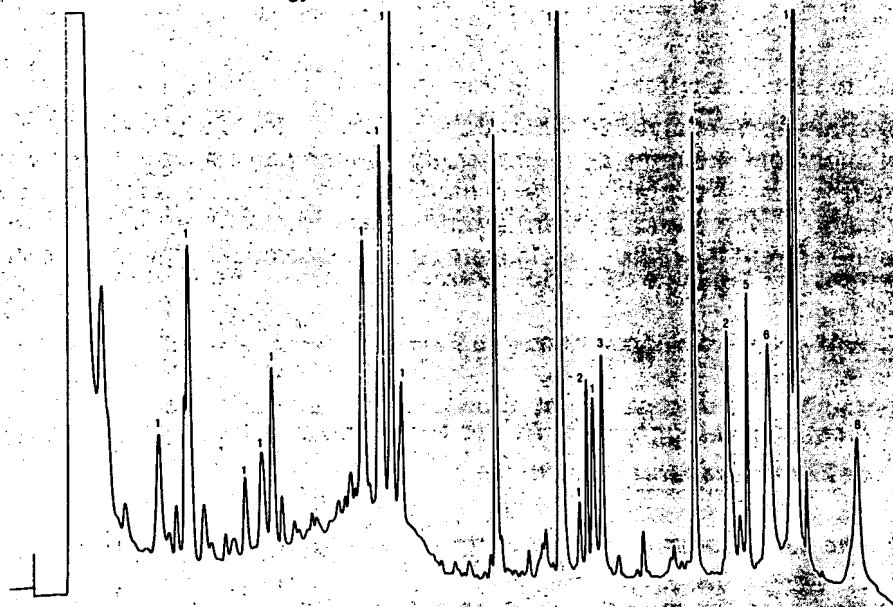


## CONDITIONS

Solvent a: .1% TFA in H<sub>2</sub>O  
 b: .1% TFA in 30/70 H<sub>2</sub>O/ACN  
 Gradient: 5% to 100% B over 30  
 Flowrate: 1.5 ml/min

## Unpublished Data

Courtesy of: Dr. Paul Tempst  
Division of Biology  
Cal Institute of Technology



## Peak Identity

- 1: Horse cytochrome c peptides
- 2: Whale myoglobin CNBr fragments
- 3: Ribonuclease
- 4: Cytochrome c
- 5: Lysozyme
- 6: BSA
- 7: Myoglobin
- 8: Ovalbumin

## Protein/Peptide Mixture

Solvent a: 0.1% TFA

Solvent b: 0.1% TFA in 70:30 ACN:H<sub>2</sub>O

Gradient: 0-50% 50 min  
50-70% 10 min  
70-100% 7.5 min

Vydac 219 TP is available in pre-packed columns and in bulk:

## COLUMNS:

Catalog No.	Particle Size	Column i.d.	Length	Price
<b>ANALYTICAL</b>				
219TP5415	5micron	4.6 mm	15 cm	\$ 300
219TP54	5 micron	4.6 mm	25 cm	325
219TP10415	10 micron	4.6 mm	15 cm	225
219TP104	10 micron	4.6 mm	25 cm	248
<b>PREPARATIVE</b>				
219TP510	5 micron	10 mm	25 cm	800
219TP1010	10 micron	10 mm	25 cm	600
219TP1022	10 micron	22 mm	25 cm	1600
219TP152022	15-20 micron	22 mm	25 cm	995

## BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
219TPB10	10 micron	19.30	15.00	12.00	11.00
219TPB1520	15-20 micron	---	3.00	3.00	2.00
219TPB2030	20-30 micron	---	2.50	2.00	1.50

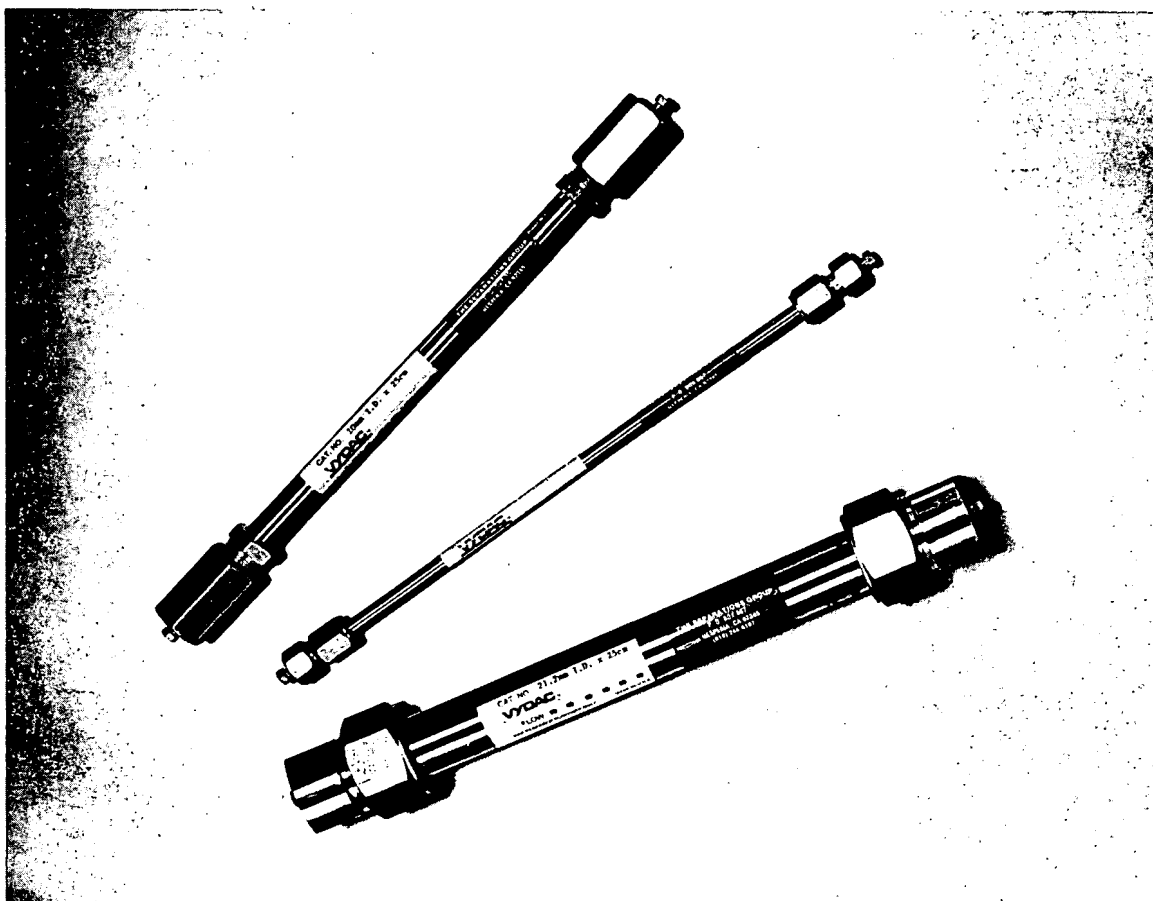
Bulk packing materials are available in larger quantities or other particle size ranges on special request.  
Ask for a quotation on price, delivery and minimum order quantity.

# Preparative HPLC Separations

For simple scale-up of analytical separations **Vydac** columns are available in three diameters: analytical (4.6 mm), semi-preparative (10 mm) and preparative (22 mm). Large diameter columns contain the same material as analytical columns and permit use of the same solvents and gradient profile. The only parameter that must be changed is the solvent flow rate. This is increased to accomodate the larger column volume.

**Vydac** preparative columns feature:

- ***Increased throughput of five to twenty times with 10 mm semi-preparative or 22 mm preparative columns***
- ***The same column packing materials are used in analytical columns***
- ***Moderate backpressure at normal operating flow rates***
- ***Direct scaleup of analytical separations by retaining both the solvents and gradient profile used with an analytical column while increasing the flow rate***



See individual product ordering information for the catalog numbers and prices of available semi-preparative and preparative columns.

**Vydac** column packing materials are offered in a range of particle sizes to allow simple scale-up of separations achieved on analytical or semi-preparative columns. **Vydac** bulk packing materials feature:

- **Three particle size ranges for maximum flexibility: a narrow distribution ten micron material, a 15-20 micron material and a 20-30 micron material**
- **The same silica substrate except for particle size and the same bonded phases as small micron analytical column packing materials**
- **Simple column packing procedures for 15-20 and 20-30 micron materials**
- **Low packpressure requirements at normal operating flow rates**
- **Little loss in resolution scaling up to larger particle size column packing materials.**

### COMPARISON OF PROTEIN SEPARATION ON DIFFERENT PARTICLE SIZE PACKING MATERIALS

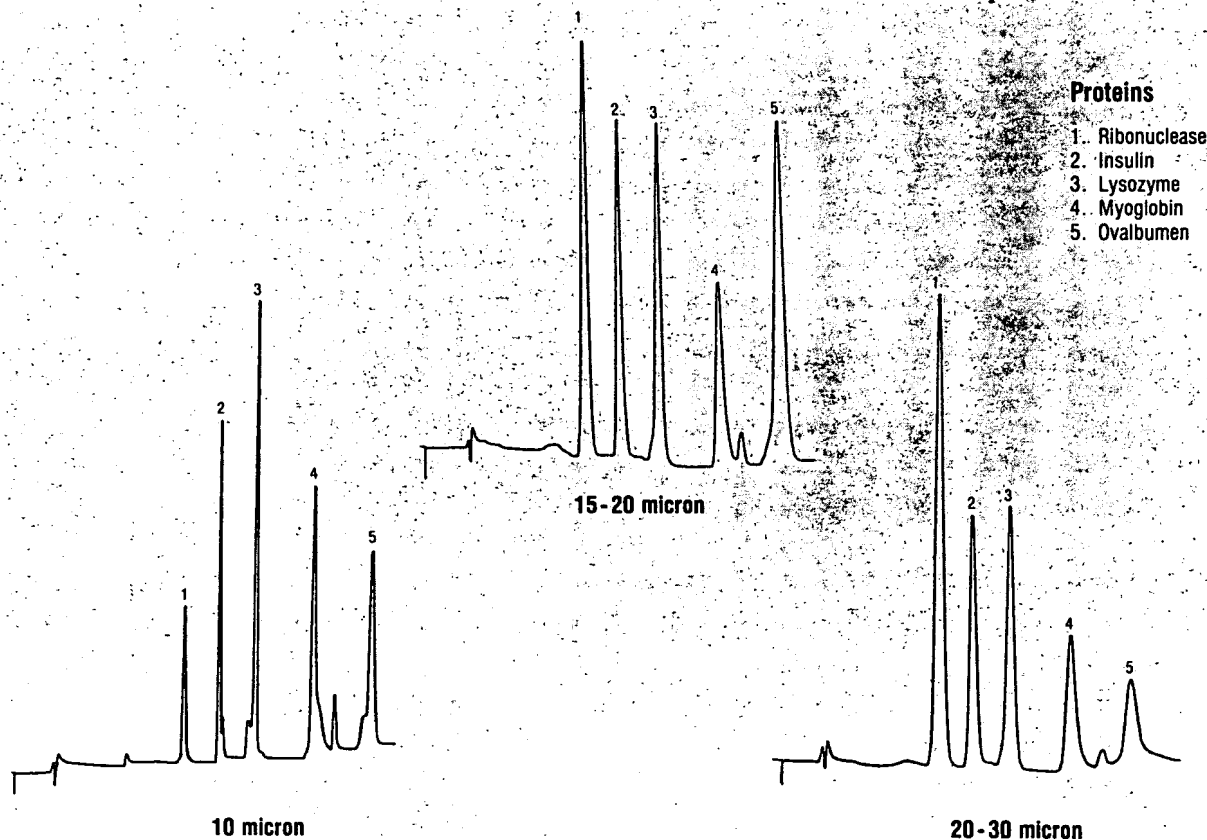
#### CONDITIONS

Material: Vydac 218 TP  
Sample: Protein Standard

Eluent: .1% TFA in 25:75 ACN:H<sub>2</sub>O to  
.1% TFA in 95:5 ACN:H<sub>2</sub>O

#### Proteins

1. Ribonuclease
2. Insulin
3. Lysozyme
4. Myoglobin
5. Ovalbumin



See individual products for prices, catalog number, and quantity discounts on bulk packing material.



# Vydac Ion Chromatography Columns

Vydac columns for non-suppressed ion chromatography set a new standard of performance for single column ion analysis by HPLC. Vydac 300IC and 400IC columns feature a small particle, low surface area silica that has been protected with a proprietary chemical treatment developed by the Separations Group permitting these columns to be used above pH 7. These low capacity ion exchange columns have been specifically designed for the separation of ions and feature:

- **Single column technology allowing use of standard HPLC components**
- **A highly efficient silica substrate**
- **Use of eluents as high as pH 9 with no column degradation**
- **Rapid analysis (2-5 minutes) with excellent resolution**
- **High sensitivity (typically sub-ppm detectability for most ions)**
- **Also available is the 302IC column for anion analysis. Although not stable in basic eluents, this column remains a standard in single column ion analysis.**

## Anion Analysis

Column: Vydac 300IC405  
Eluent: 1.5 mM phthalate  
pH 8.9

### Peak Identity

1. Solvent
2.  $F^-$  5 ppm
3.  $CO_3^{2-}$  dissolved
4.  $Cl^-$  1 ppm
5.  $NO_2^-$  1.5 ppm
6.  $Br^-$  3 ppm
7.  $NO_3^-$  2.5 ppm
8.  $HPO_4^{2-}$  3 ppm
9.  $SO_4^{2-}$  3 ppm



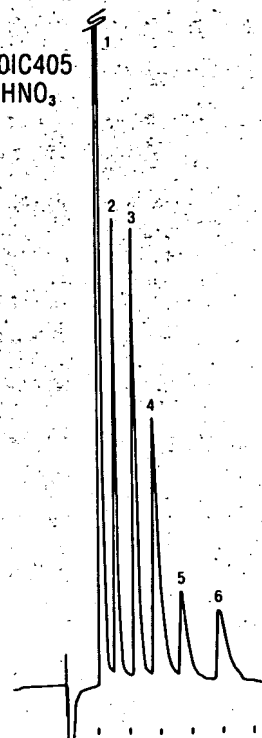
4 min.

## Cation Analysis

Column: Vydac 400IC405  
Eluent: 2.5 mM  $HNO_3$

### Peak Identity

1.  $Li^+$
2.  $Na^+$
3.  $NH_4^+$
4.  $K^+$
5.  $Rb^+$
6.  $Cs^+$



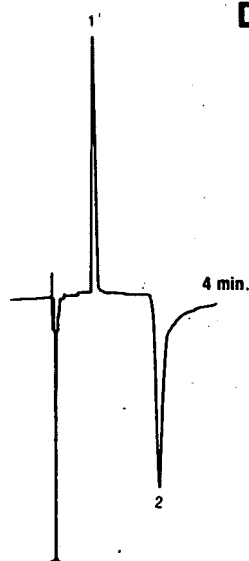
6 min.

### Dichromate

Column: Vydac 300IC405  
Eluent: 3 mM phthalic acid  
pH 8.0

#### Peak Identity

1. Sulphate
2. Dichromate

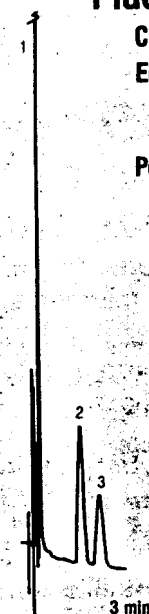


### Fluorophosphate

Column: Vydac 300IC405  
Eluent: 2.5 mM phthalic acid  
pH 8.6

#### Peak Identity

1. Fluoride
2. Phosphate
3. Fluorophosphate

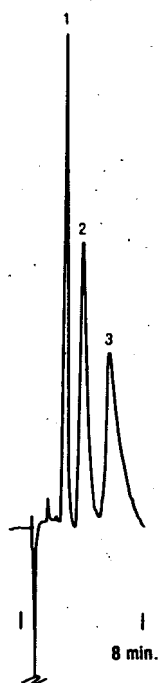


### Amines

Column: Vydac 400IC405  
Eluent: .01 M H<sub>3</sub>PO<sub>4</sub>  
+ 10% MeOH

#### Peak Identity

1. Methyamine
2. Dimethylamine
3. Trimethylamine

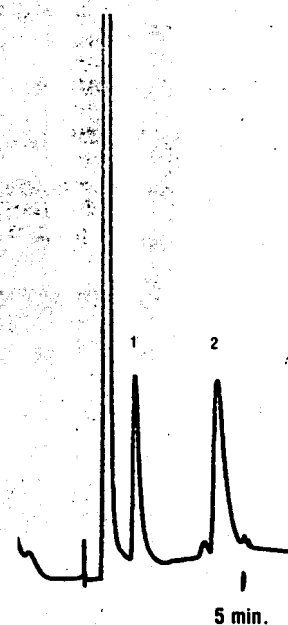


### Organic Acids

Column: Vydac 302IC4.6  
Eluent: 1 mM Phthalic Acid  
pH 4.6

#### Peak Identity

1. Acetate
2. Formate



Vydac Ion Chromatography materials are available only in pre-packed columns.

Catalog No.	Type	Column i.d.	Length	Price
300IC405	Anion	4.6 mm	5 cm	\$ 350
302IC4.6	Anion	4.6 mm	25 cm	240
400IC405	Cation	4.6 mm	5 cm	350

# Vydac Ion Exchange Columns and Packing Materials

Vydac microparticulate ion exchange materials are available with both anion and cation exchange groups. These are chemically bonded to ten micron low surface area TP silica. Vydac microparticulate ion exchange materials include:

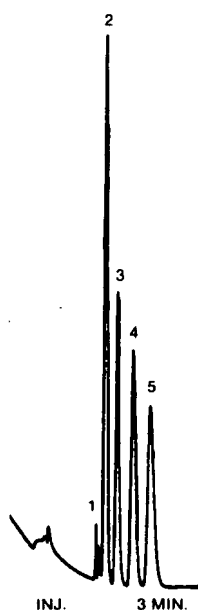
- **301 TP, a strong anion exchange material with a quaternary amine functional group for the analysis of nucleotides and other organic acids**
- **401 TP, a strong cation exchange material with a sulphonic acid functional group for analysis of amines, purines, pyrimidines and other organic bases.**

## 301 TP

### NUCLEOTIDES

1. Solvent
2. CMP
3. AMP
4. UMP
5. GMP

Eluent:  
.05 M  $\text{NaH}_2\text{PO}_4$   
pH 2.79

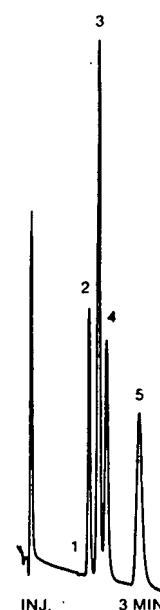


## 401TP

### NUCLEOSIDES

1. Solvent
2. Uracil
3. Cytosine
4. Hypoxanthine
5. 5-Methyl cytosine

Eluent:  
.1 N  $\text{NH}_4\text{OAc}$   
pH 5.27



301 TP and 401 TP ion exchange materials are available in pre-packed columns and in bulk:

### COLUMNS:

Catalog No.	Type	Column I.d.	Length	Price
301TP104	Anion	4.6 mm	25 cm	\$ 264
401TP104	Cation	4.6 mm	25 cm	264

### BULK:

Catalog No.	Type	Price (10 gm)
301TPB10	Anion	\$218.00
401TPB10	Cation	218.00

Note: request quotation for larger quantities of bulk.

# Vydac Solid Core Materials and Pre-column Kits

**Vydac SC (Solid Core)** is a unique separation material made by chemically coating a solid glass support with silica to which various phases are chemically bonded.

**Vydac SC** has a surface area of 8 sq. m./gram and a particle size distribution centered at 30 microns. Generally used for pre-columns, **Vydac SC** materials feature:

- *Free flowing, high density material for easy packing of columns*
- *Thermally and hydrolytically stable material for reliable, extended use*
- *Available in bulk and in pre-column kits*

## BULK SOLID CORE

Catalog No.	Material	Price (25 grams)
101SC	Silica adsorbent	\$ 129.00
201SC	Reverse phase (C <sub>18</sub> )	184.00
301SC	Anion exchange	216.50
401SC	Cation exchange	216.50
501SC	Polar (CN) phase	184.00
601SC	Polar (NH <sub>2</sub> ) phase	184.00

## PRE-COLUMN KITS

Catalog No.	Material	Price
101PSC	Silica adsorbent	\$ 76.00
201PSC	Reverse phase (C <sub>18</sub> )	92.00
301PSC	Anion Exchange	102.00
401PSC	Cation exchange	102.00
501PSC	Polar (CN) phase	92.00
601PSC	Polar (NH <sub>2</sub> ) phase	92.00

Pre-column kits contain the necessary hardware to make a guard column and seven grams of the designated solid core material, sufficient to fill the guard column three times.

# **Vydac Selected Bibliography**

## **Small Hydrophobic Molecules**

1. "Liquid Chromatographic Determination of Polycyclic Aromatic Hydrocarbons in Air Particulate Extracts"  
May, W. E. Wise, S.A.  
Anal Chem 56 (2) 225-232 (1984)
2. "Development of Sampling and Analytical Methods for Toxicants in Diesel Exhaust Streams"  
Arthur D. Little Inc., Mar 15, 1983  
Prepared for the Bureau of Mines, Washington, D.C.  
PB84-196625
3. "High Performance Liquid Chromatographic Separation of High Molecular Weight Polycyclic Aromatic Compounds in Carbon Black."  
Peadar, P. A., Lee, M. L., Hirata, Y., Novotny, M.  
Anal Chem 52(14) 2268-2271 (1980)
4. "A Novel Integrative Technique for Locating and Monitoring Polynuclear Aromatic Hydrocarbon Discharges to the Aquatic Environment"  
Black, J. J., Hart Jr., T. F., Black, P. J.  
Environ Sci and Tech 16 (5) 247-250 (1982)
5. "Summertime Variations in Polycyclic Aromatic Hydrocarbons at Four Sites in New Jersey"  
Harkov, R., Greenberg, A., Darack, F., Daisey, J. M.  
Environ Sci and Tech 18 (4) 287-291 (1984)

## **Ion Chromatography**

1. "Design and Performance of a Modular Chromatograph for Chromatography of Ions"  
Stevenson, R. L., Harrison, K.  
Amer Lab 13(5) 76-81
2. "Analysis of Atmospheric Aerosols by Nonsuppressed Ion Chromatography"  
Willison, M. J., Clarke, A. G.  
Anal Chem 56 (6) 1037-1039 (1984)
3. "Optimization of Anion Separation by Nonsuppressed Ion Chromatography"  
Jenke, D. R., Pagenkopf, G. K.  
Anal Chem 56 (1) 85-88 (1984)
4. "Standardization of Transparent Analyte Response in Indirect Photometric Chromatography"  
Jenke, D. R.  
Anal Chem 56 (13) 2468-2470 (1984)

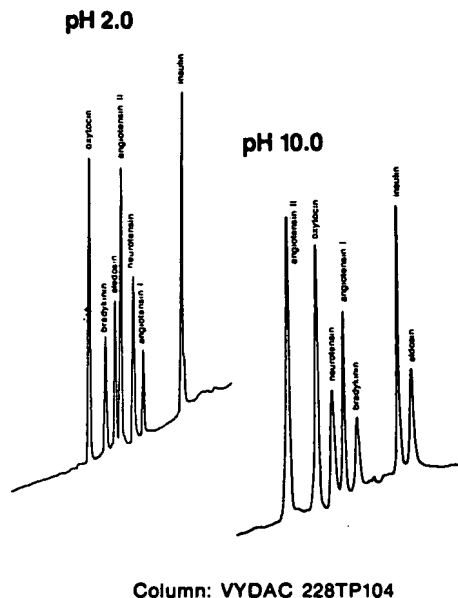
## Proteins and Peptides

1. "The Importance of Silica Type for Reverse-Phase Protein Separations"  
J. D. Pearson, N. T. Lin and F. E. Regnier  
Anal. Biochem. 124, 217-230 (1982)
2. "Reversed-Phase High-Performance Liquid Chromatography: Preparative Purification of Synthetic Peptides"  
J. Rivier, R. McClintock, R. Galyean and H. Anderson  
J. of Chromatog. 288, 303-328 (1984)
3. "Reversed-Phase High Performance Liquid Chromatography of Insulins from Different Species"  
J. Rivier and R. McClintock  
J. of Chromatog. 268, 112-119 (1983)
4. "Reversed-Phase HPLC Separation of Somatic Histones"  
J. A. Mazrimas and R. Balhorn  
Liquid Chromatography Vol 1, No. 2 p 104-105 (1983)
5. "High Performance Liquid Chromatographic Separation of Globin Chains on a Large-Pore C<sub>4</sub> Column"  
J. B. Shelton, J. R. Shelton and W. A. Schroeder  
J. Liquid Chrom. 7 (10), 1969-1977 (1984)
6. "High Performance Liquid Chromatography of Proteins as Applied to Fibrinogen Chains"  
M. Kehl, F. Lottspeich and A. Henschen  
Z. Physiol. Chem 363, 1501-1505 (1982)
7. "Sequence Analysis of a Growth Hormone Releasing Factor from a Human Pancreatic Islet Tumor"  
Spiess, J., Rivier, J., Thorner, M., Vale, W.  
Biochem 21 (24) 6037-6040 (1982)
8. "Synthesis of Potent Heptapeptide Analogues of Cholecystokinin"  
Penke, B., Hahnal, F., Lonovics, J., Holzinger, G., Kadar, T., Telegdy, G., Rivier, J.  
Journ of Med Chem 27 (7) 845-849 (1984)
9. "Reverse-Phase High-Performance Liquid Chromatography of Hydrophobic Proteins and Fragments Thereof"  
Tarr, G. E., Crabb, J. W.  
Anal Biochem 131 (1983) 99-107
10. "Application of High-Performance Liquid Chromatographic Techniques to the Separation of Ribosomal Proteins of Different Organisms"  
Kamp, R. M., Bosserhoff, A., Kamp, D., Wittman-Liebold, B.  
J Chrom 317 (1984) 181-192

# NEW HPLC Products from VYDAC!

**VYDAC pH Stable Reverse Phase Columns** are C-8 columns based on VYDAC wide pore TP silica and are stable from pH 2 to 10. pH stable reverse phase columns are ideal for chromatographing biomolecules at neutral or basic pH and add a new dimension to the separation of peptides.

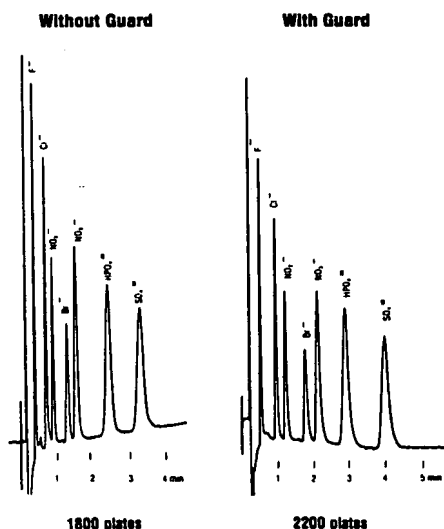
AFFECT OF pH ON RELATIVE RETENTION OF PEPTIDES



**VYDAC High Performance Guard Columns** are packed with the same material as analytical columns. High Performance Guard Columns fit directly on top of VYDAC analytical columns and can actually enhance performance while increasing column lifetime.

AFFECT OF GUARD COLUMN ON ANALYTICAL COLUMN PERFORMANCE

Column: VYDAC 300IC405

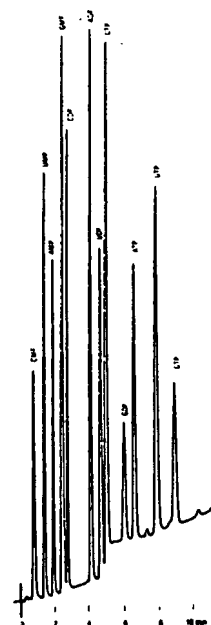


**The VYDAC Nucleotide Analysis Column** can separate the twelve major nucleotides in 10 minutes.

SEPARATION OF MAJOR NUCLEOTIDES

Column: VYDAC 303NT405

- 1 - CMP
- 2 - UMP
- 3 - AMP
- 4 - GMP
- 5 - CDP
- 6 - ADP
- 7 - UDP
- 8 - CTP
- 9 - GDP
- 10 - ATP
- 11 - UTP
- 12 - GTP



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